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PRINCIPAL INVESTIGATOR: Paulette Furbert-Harris, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Washington, DC 20059

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Paulette Furbert-Harris, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Howard University
Washington, DC 20059

e-mail:
pfurbert-harris@fac.howard.edu

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13. ABSTRACT ***(Maximum 200 Words)***

We have demonstrated that cell lines developed from metrizamide density fractions of peripheral blood hypodense (.22) and hyperdense (.24) eosinophils significantly inhibited LNCaP, PC3, DU145 and HPC1 monolayer growth cultures in vitro. This activity was enhanced when the eosinophils were pretreated with interleukin-5. DU145 and PC3 colony formation was inhibited 50-75% by eosinophil cell lines, while peripheral blood eosinophils inhibited PC3 colony formation by as much as 95%. 24hr. cultured eosinophil supernatants significantly inhibited PC3 colony formation with 3/7 supernatants causing 100% inhibition. Inhibitory activity of the 24hr. supernatants towards DU145 was more variable, ranging from 9-90% inhibition of colony formation. There was slight enhancement of growth with one preparation. IL-4 and TNFα, which were found present in the supernatants also inhibited growth of monolayers cultures of PC3, DU145 and HPC1. These data strongly support the hypothesis that eosinophils can destroy prostate tumor cells in vitro. The eosinophil cell lines and cytokine modulation of their activity offer an exquisite tool for more detailed study (both cellular and molecular) of a role for eosinophils as anti-prostate cancer effector cells.

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FOREWORD

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Paulette Turbeville-Harris 1/5/00

PI - Signature Date

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5. Introduction

Prostate cancer is the most common cancer diagnosed in American men. It has been estimated that by the end of 1999, 179,000 men will have been diagnosed with prostate cancer and that 37,000 deaths will have resulted(1). Prostate cancer incidence and mortality rates for African American males are the highest of any racial or ethnic group in the world (2). Prostate cancer incidence in this group is 180.6/100,000, compared to 143.7/100,000 for Caucasians and 24.2/100,000 for Koreans (2). The mortality rate for African American is 53.7 compared to 24.1 for Caucasians and 6.6 for Chinese.

Several new treatment approaches towards the eradication of prostate cancer have focused on regulating the immune response system to antigens expressed on prostate cancer cells (3-7). Moreover the strategy of utilizing cytokine gene therapy in order to amplify the host response to tumor is quickly gaining momentum. Many of the cytokines which have been used (e.g. IL-2, IL-4, IL-5 and GM-CSF) are known to either attract and/or regulate eosinophil activity(8).

Eosinophils have been traditionally known as anti-helminthic effector cells and inflammatory agents in hypersensitivity reactions, particularly allergic asthma(9). Evidence exists, however, for a potential role for eosinophils in cancer. We have recently shown that activated eosinophils destroy MCF-7 and MDA-231 breast cancer cell monolayer formation in vitro and inhibit MCF-7 colony formation (manuscript in preparation). The inhibition observed is partially mediated by cytokines IL-4 and TNFα which were secreted into 24-hr conditioned supernatants. In this study, we have examined the inhibitory activity of activated eosinophils and eosinophilic cell lines which we had previously established and are presently characterizing (manuscript in preparation) on prostate cancer cell lines in eosinophil:tumor co-culture assays, and also the effect of cultured eosinophil supernatants on cell growth.

6. Body

Propagation of Cell Lines: To date all six eosinophilic cell lines have been retrieved from storage at -160°C, cultured in RPMI medium supplemented with penicillin/streptomycin (50 units/50 ug/ml, respectively), gentamycin (50ug/ml) and 10% fetal bovine serum. At present we have data with 3 of the cell lines and 2 sublines. **Tumor**: PC-3, DU145 and LNCaP cells were obtained from ATCC and established in culture, frozen and retrieved prior to use. They are being maintained in the appropriate culture medium as recommended by the vendor, PC3 (7% F-12K medium supplemented with penicillin/streptomycin and gentamycin). In a collaborative study, we at Howard University have very recently immortalized a primary prostate culture HPC1 from an African American which is presently being characterized. These cells are also cultured in 10% RPMI medium containing penicillin/streptomycin and gentamycin and were used preliminarily in this study.

Growth Inhibition of PC3, LNCaP, DU145 and HPC1 Tumor Cells by Activated Eosinophils and Eosinophilic Cell Lines.

A. Monolayer. Tumor Cells (PC3, LNCaP, DU145 and HPC1 were seeded into the wells of either a 6-well plate (at $2.5x10^5$ cells per well) or a 12-well plate (at $1.5x10^5$ cells per well). The plates were incubated overnight (16-24hr) at 37°C, 5% CO_2. Eosinophils were added at various effector to target (E:T) ratios and the plates were incubated for an additional 24-48hr. The effector cells were then removed, the monolayers washed 3x with PBS and stained with hematoxylin and eosin before being photographed. As another form of documentation, the entire plate was scanned into power point and is represented.

LNCaP was extremely sensitive to both hypodense eosinophils at 5:1 and 43:1 E:T ratios (Fig. 1B and 1C, respectively) and hyperdense eosinophils at 5:1 and 14:1 E:T ratios (Fig. 1D and 1E, respectively). At the same E:T ratios, PC3 was also sensitive to killing by eosinophil hypodense and hyperdense cell lines (Fig. 2). Moreover, eosinophil cell lines established from both hypodense and hyperdense eosinophil fractions from another donor also inhibited PC3 cell growth at the E:T ratio of 10:1 (Fig. 3B- hypodense cell line; 3E-hyperdense cell line), and HPC1 cell growth also at 10:1 E:T ratio (Fig. 11B) . IL-5 pretreatment of the eosinophils resulted in even greater killing of both PC3 (Fig. 3C and 3F) and HPC1 (Fig. 11C). The entire 12-well plates were scanned in an effort to get a more comprehensive evaluation of tumor cell damage (Fig. 4), (Fig. 12).

Eosinophil cell lines were sterile sorted with a Becton Dickinson FACS SCAN Cell Sorter using the PE-labelled antibody to the eotaxin receptor. This chemokine receptor is found predominantly on eosinophils. These sublines were found also to be positive for the eosinophil markers CD15 and CD49d. Both the parent eosinophil cell line GRC.014.24 and the two sublines GRC014.24.S1 and GRC.014.24.S2 markedly inhibited PC3 cell growth (Fig. 5).

When 24hr. cultured eosinophil supernatants were added to subconfluent PC3 (Fig. 6), DU145 (Fig. 9) and HPC1 (Fig. 13) monolayer cell growth was dramatically inhibited except for donor BLA who's eosinophil supernatants showed little damage to the PC3 monolayer, but completely destroyed both DU145 and HPC1 monolayers.

B. Colony Formation. PC3 and DU145 cells were seeded into the wells of 6-well tissue culture plates at 100 cells per well. The plates were incubated overnight at 37°C, 5% CO_2. At this time effector cells were added at various E:T ratios and the plates were incubated for ten days. The plates were harvested, washed with PBS 3x, then stained with hematoxylin and eosin and counted manually. Both hypodense and hyperdense subpopulations of peripheral blood eosinophils inhibited PC3 colony formation in a dose-dependent manner, (Fig. 7A), with the 50:1 E:T ratio resulting in 95% inhibition for the hypodense eosinophils and 91% inhibition for both 10:1 and 50:1 E:T ratios for the hyperdense eosinophils. The cell line GRC.014.24 inhibited colony formation by 71 and 75% at E:T ratios 1:2 and 2:1, respectively, while the subline GRC.014.24.S1 had minimal effect at both E:T ratios (7% and 18%, respectively). At the E:T ratio of 2:1, GRC.014.24 inhibited DU145 by 88% and the sublines Sl and S2 inhibited colony formation by 8l and 54%, respectively (Fig. 10A). The hypodense cell line BJA.060.22 inhibited colony formation by 50%.

C. 24hr. Cultured Supernatants Inhibit Prostate Tumor Cell Growth In Vitro. Subconfluent monolayers of PC3, DU145 and HPCl prostate cells were incubated overnight with 24-hr. cultured supernatants in 12-well tissue culture plates. Both hypodense and hyperdense eosinophil cultured supernatants markedly inhibited PC3 colony formation (Fig. 7C) and at least three supernatant

preparations (BLA 24, HMO 22 and HMO 24) completely prohibited colony formation. On the otherhand DU145 was less sensitive to many of the supernatant preparations, particularly BLA 24, HMO 22, YDA 22 and WCH 22, (Fig. 10B). GRC.014.24 supernatant completely inhibited DU145 colony formation (Fig. 10C).

D. Cytokine Presence in 24hr. Eosinophil Supernatants. 24hr. cultured supernatants from peripheral blood eosinophil hypodense and hyperdense subpopulations (M22 and M24, respectively) were evaluated by enzyme-linked immunoassay (ELISA) analysis using commercial kits. Interleukin-4 (IL-4) and Tumor Necrosis Factor Alpha (TNFα) were present in varying levels in all individuals tested (Table I). IL-4 concentrations ranged from 0 to >1000 pg/ml. TNFα concentrations were far less than IL-4, ranging from 10-224pg/ml. GM-CSF was only found in donor 6 supernatants at 450pg/ml, and IL-5 was absent in all 6 samples.

7. Key Research Accomplishments

- Retrieval of all eosinophilic cell lines
- Demonstration of functional cytotoxic/cytostatic activity with 3 of the lines and 2 sublines
- Establishment of new prostate cell line in collaboration with clinical investigators at Howard University Hospital
- Use of new prostate cell line in eosinophil co-culture assays.

8. Reportable Outcomes

Activated eosinophils inhibit cell in vitro growth of prostate cancer cell lines, (Manuscript in preparation)

Late Abstract in preparation for AACR Spring 2000

Ahaghotu C, Marshalleck J, Dennery M, Vaughn T, Laniyan I, Jackson A, **Furbert-Harris P**. A Novel Primary Prostate Cancer Cell Line Derived from an African American Patient. The American Urological Association, Inc. 95th Annual Meeting, 1999.

9. Discussion/Conclusions

We hypothesized that activated eosinophils which may be found in tumor infiltrates produce cytokines which are both tumor inhibitory and enhancing. Moreover these cytokines may modify adhesion molecule expression on tumor cells thereby modifying their mortality and metastatic capabilities. The tasks of the first 12-months entailed:

a. culturing and propagation of both prostate cells and eosinophilic cell lines.
b. growth inhibition assays (monolayer/colony).
c. cytokine enhancement of eosinophil activity.
d. exogenous cytokine activity against prostate tumor cell growth.

The data presented have clearly shown that subpopulations of activated eosinophils, (hypodense and hyperdense) from individuals with mild to moderate eosinophilia inhibit the growth of PC3, tumor cells (both monolayer and colony formation). Furthermore eosinophil cell lines established from these subpopulation inhibited LNCaP, PC3, DU145 and the newly established prostate cell line HPC1. In the colony assay PC3 was more sensitive than DU145, to eosinophil killing. IL-5 which is known to activate eosinophils, when used to pretreat both hypodense and hyperdense cell lines, enhanced their killing of both PC3 and HPC1 tumor cells.
Both LNCaP and HPC1 failed to form colonies and hence we simply used monolayers to test eosinophil activity. IL-5 has been the only cytokine used thus far to enhance eosinophil activity. We will continue to study this with other cytokines known to stimulate eosinophils (e.g. IL-3, GM-CSF, IL-4). ELISA analysis of eosinophil supernatants revealed the presence of IL-4, TNFα and GM-CSF.

We will continue to characterize the supernatants for other cytokines. In addition to completing the tasks for year one, we have already begun to assess the baseline presence of adhesion molecules on the tumor cells and will begin with the cytokine modulation of these in the very near future.

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11. Apendices.

Figure 1. Inhibition of LNCaP Tumor Cell Growth by Eosinophil Cell Lines

Figure 1. Legend

Figure 2. Inhibition of PC3 Tumor Cell Growth by Eosinophil Cell Lines

Figure 2. Legend

Figure 3. IL-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro

Figure 3. Legend

Figure 4. IL-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cell In Vitro

Figure 4. Legend

Figure 5. CD15 and CD49d Positive Eosinophil Sublines Inhibit PC3 Cell Growth In Vitro

Figure 5. Legend

Figure 6. 24-hr. Cultured Eosinophil Supernatants Inhibit PC3 Cell Growth In Vitro

Figure 6. Legend

Figure 7. Eosinophils Inhibit PC3 Colony Formation In Vitro

Figure 7. Legend

Figure 8. Eosinophil Cell Lines Inhibit DU145 Cell Growth In Vitro

Figure 8. Legend

Figure 9. 24-hr. Cultured Supernatants Inhibit DU145 Cell Growth In Vitro

Figure 9. Legend

Table 1. Cytokine Concentrations in 24hr Eosinophil Culture Supernatants (pg/ml)

Table 1. Legend

Figure 10. DU145 Colony Inhibition by Eosinophil Cell Lines and Cultured Supernatants

Figure 10. Legend

Figure 11. HPC1 Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines

Figure 11. Legend

Figure 12. HPC1 Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines

Figure 12. Legend

Figure 13. 24-hr. Cultured Supernatants Inhibit HPC1 Cell Growth In Vitro

Figure 13. Legend

Fig. 1. Inhibition of LNCaP Tumor Cell Growth by Eosinophil Cell Lines

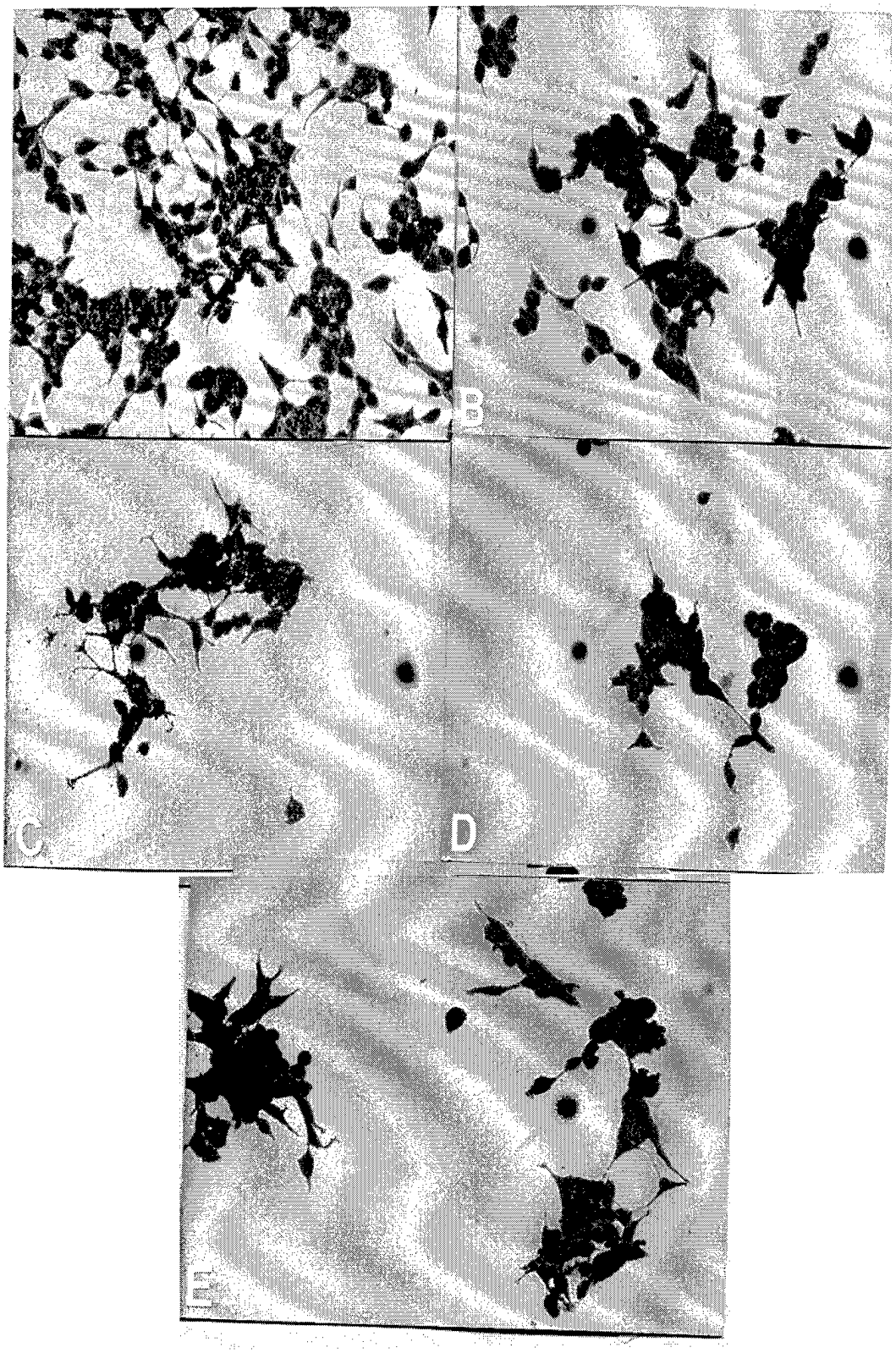


Fig. 1. LNCaP tumor cells were seeded into T25 flasks at 3×10^5 cells/flasks and allowed to grow to confluence (3-4 days) in media alone (A) or in the presence of hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B&C, respectively) and hyperdense cell line (SD.031.24) at E:T ratios of 5:1 and 14:1 (D and E, respectively). All experiments were performed in duplicate.

Fig. 2. Inhibition of PC3 Tumor Cell Growth by Eosinophil Cell Lines

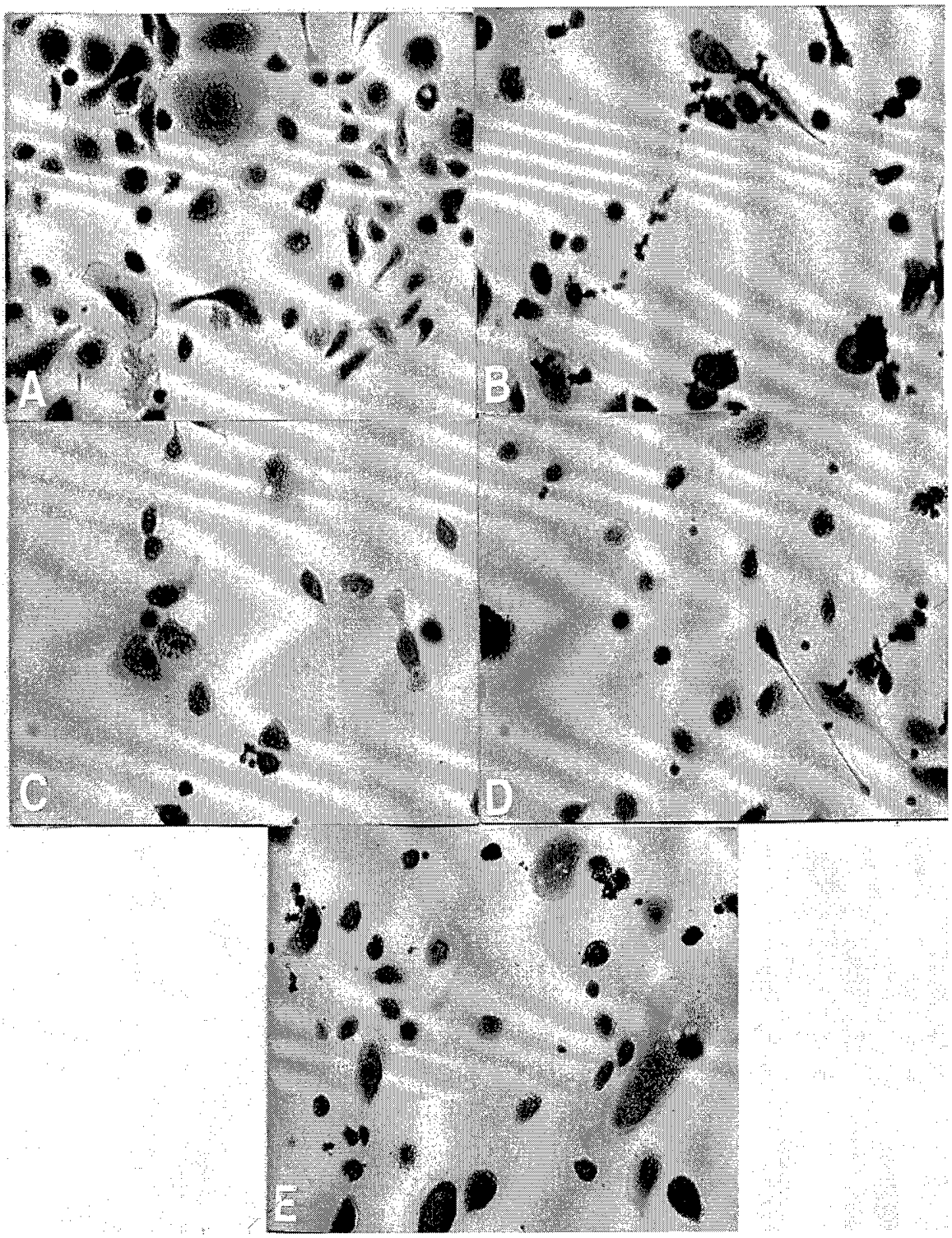


Fig. 2 PC-3 tumor cells were seeded into duplicate T25 flasks at 3×10^5 cells/flask and allowed to grow to confluence (3-4 days) in media alone, and in co-culture with hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B, C, respectively) and hyperdense cell line SD.031.24 at E:T ratios of 5:1 and 14:1 (D & E, respectively).

Fig. 3. Interleukin-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro

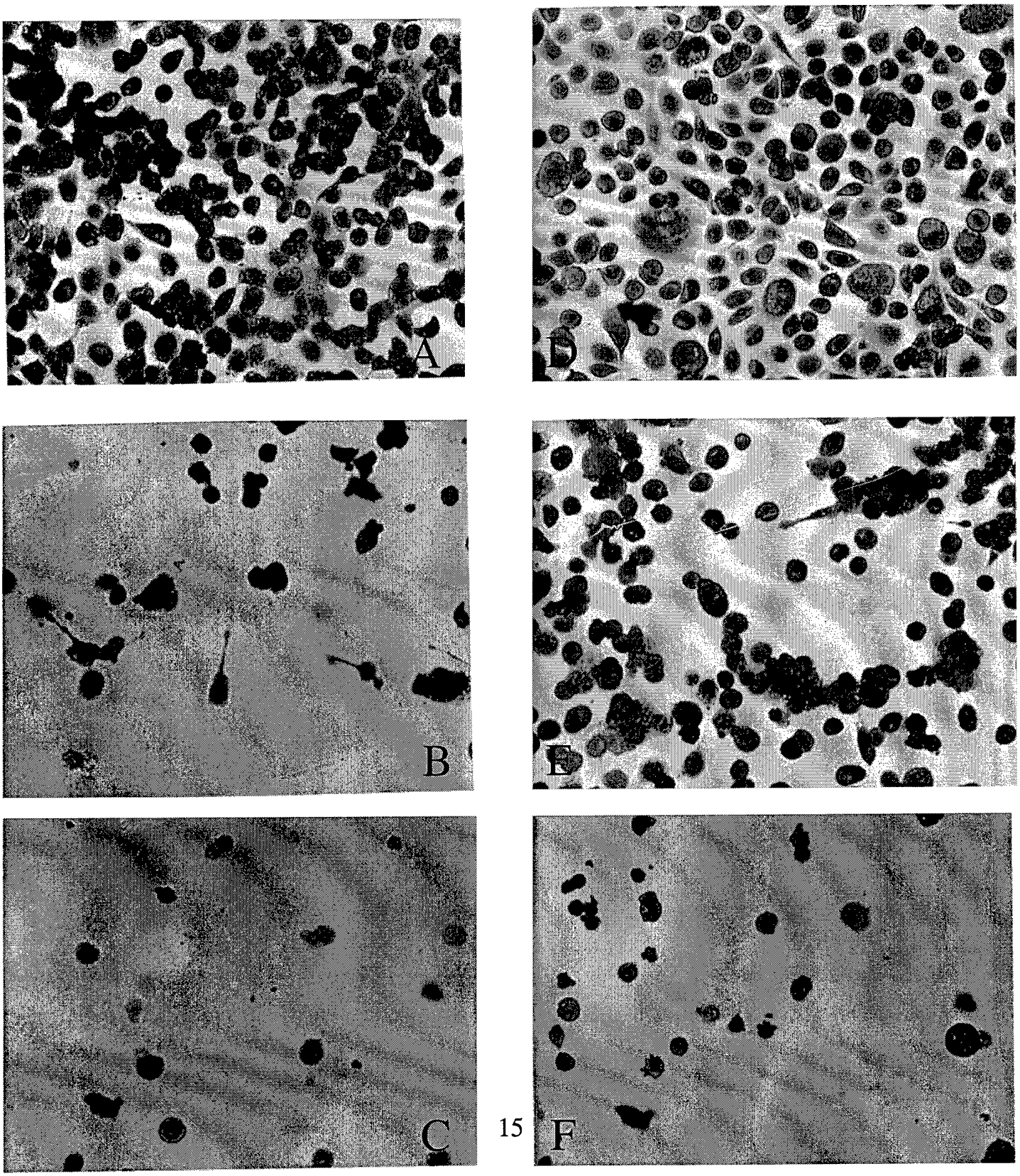
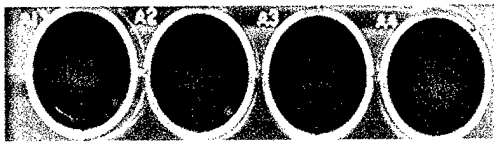


Photo Micrograph

Fig. 3. PC-3 tumor cells were seeded into the wells of a 12 well culture plate at 1.5×10^5 cells/well. Prior to this effector eosinophil cell lines were pretreated with IL-5 (l ng/ml) for 3 days. On day 4, eosinophils were added and the plate incubated for 24hr or until the control wells were confluent (24-48hrs.). Effector cells were removed and the wells washed 3X with PBS, then fixed and stained with H&E. Photomicrographs were taken as well as a scan of the entire well or plate.

Fig. 4. IL-5 Treatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro



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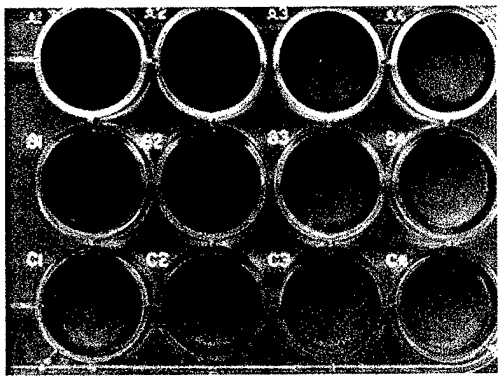
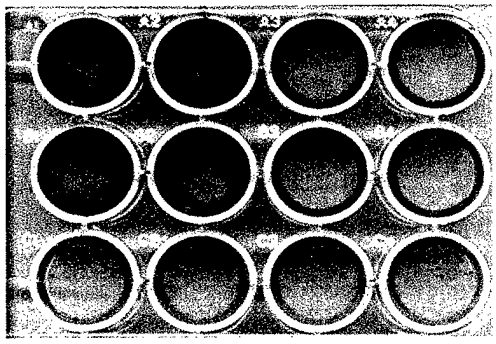


A1-2: GRC014"24":PC-3, 1:1
B1-2:GRC014"24":PC-3, 10:1
C1-2:GRC014"24":PC-3, 25:1
A3-4: GRC014"24"+IL-5:PC-3, 1:1
B3-4: GRC014"24"+IL-5:PC-3, 10:1
C3-4: GRC014"24"+IL-5:PC-3, 25:1



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B1-2:BJA060"22":PC-3, 10:1
C1-2:BJA060"22":PC-3, 25:1
A3-4: BJA060"22"+IL-5:PC-3, 1:1
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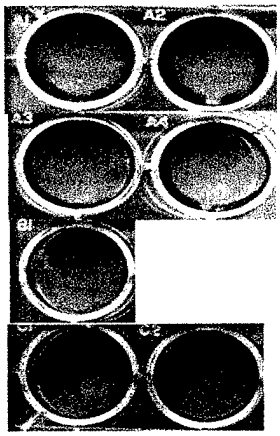


WCH"22", 5:1(Peripheral Blood Eosinophils)

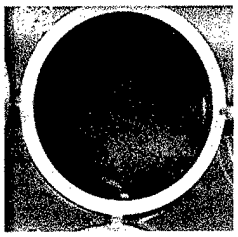
WCH"22", 10:1(Peripheral Blood Eosinophils)

WCH"22", 25:1 (Peripheral Blood Eosinophils)

WCH"24",5:1 (Peripheral Blood Eosinophils)

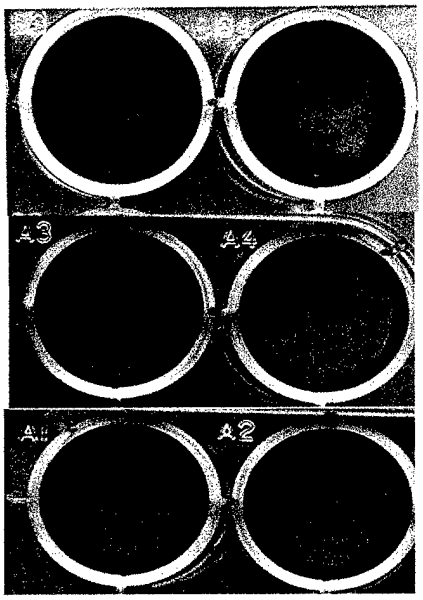
Fig. 4. Eosinophils, both peripheral blood and eosinophil cell lines were co-cultured in duplicate wells of a 12-well tissue culture plate with PC3 tumor cells at E:T ratios 1:1, 10:1 and 25:1 as described in fig. 3. The plates were harvested and stained with H & E then scanned into power point for presentation. The alpha numeric represents the donor and the numbers in quotations represent the eosinophil subpopulations (22-hypodense and 24-hyperdense).

Fig. 5. CD15 and CD49d Positive Eosinophil Sublines Inhibit PC3 Cell Growth In Vitro



Control

Panel A



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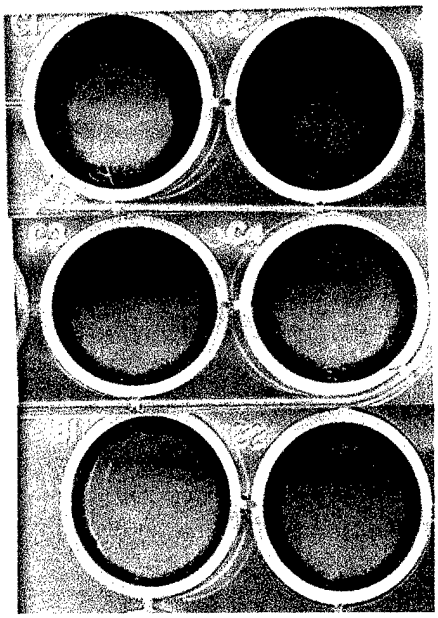
Panel B



Panel C

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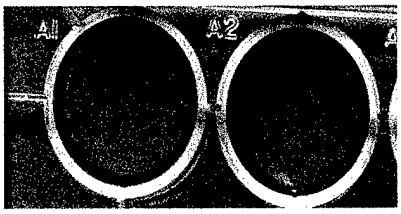
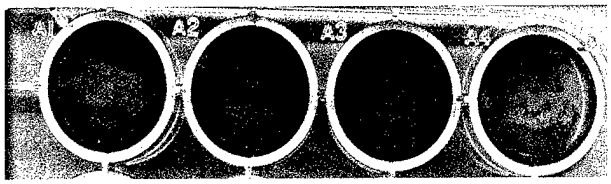


Fig. 5. Sublines from the eosinophil parent line GRC.014.24 were sterile-sorted with a FACS SCAN cell sorter using antibody to the eosinophil specific eotaxin receptor. The sublines S1 and S2 were found to be positive for both CD15 and CD49d markers. The co-culture was set up similarly to that described in figures 3 and 4. Numbers 1, 2 and 3 represent E:T ratios 1:2, 2:1 and 5:1, respectively.

.. Fig. 6 24-hr Cultured Eosinophil Supernatants Inhibits PC3 Cell Growth In Vitro



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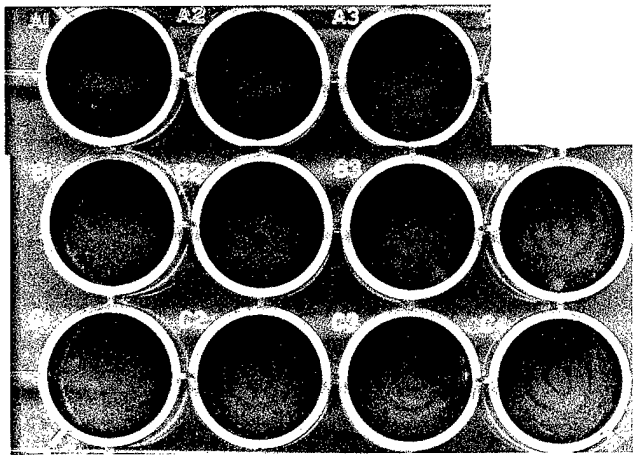
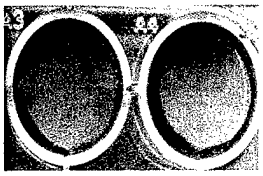
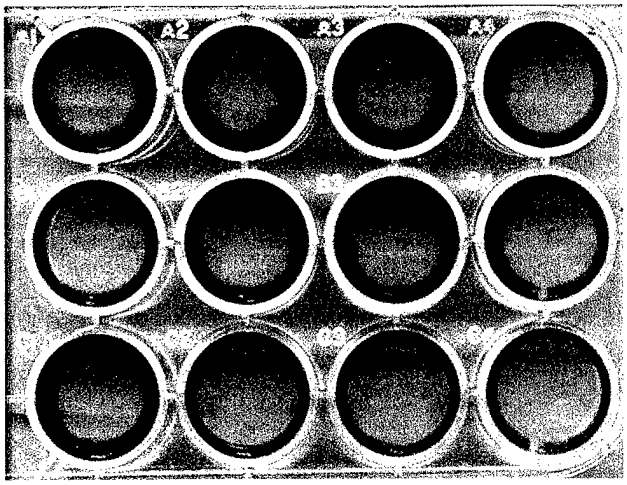


A1-2: BLA"22"
A3: BLA"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"



GRC.014.24



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 6. PC-3 tumor cells were incubated (1.5×10^5 cells/well) overnight at 37°C. Duplicate wells were then treated with 24hr. cultured supernatants from peripheral blood eosinophil hypodense (22) and hyperdense (24) subpopulations, from donors BLA, HMO, YDA and WCH. Tumor 1cells were also treated with IL-4 and TNFα at 10, 50 and 100ng/ml 24-48hr later.

Fig. 7. Eosinophil Inhibit PC-3 Colony Formation In Vitro

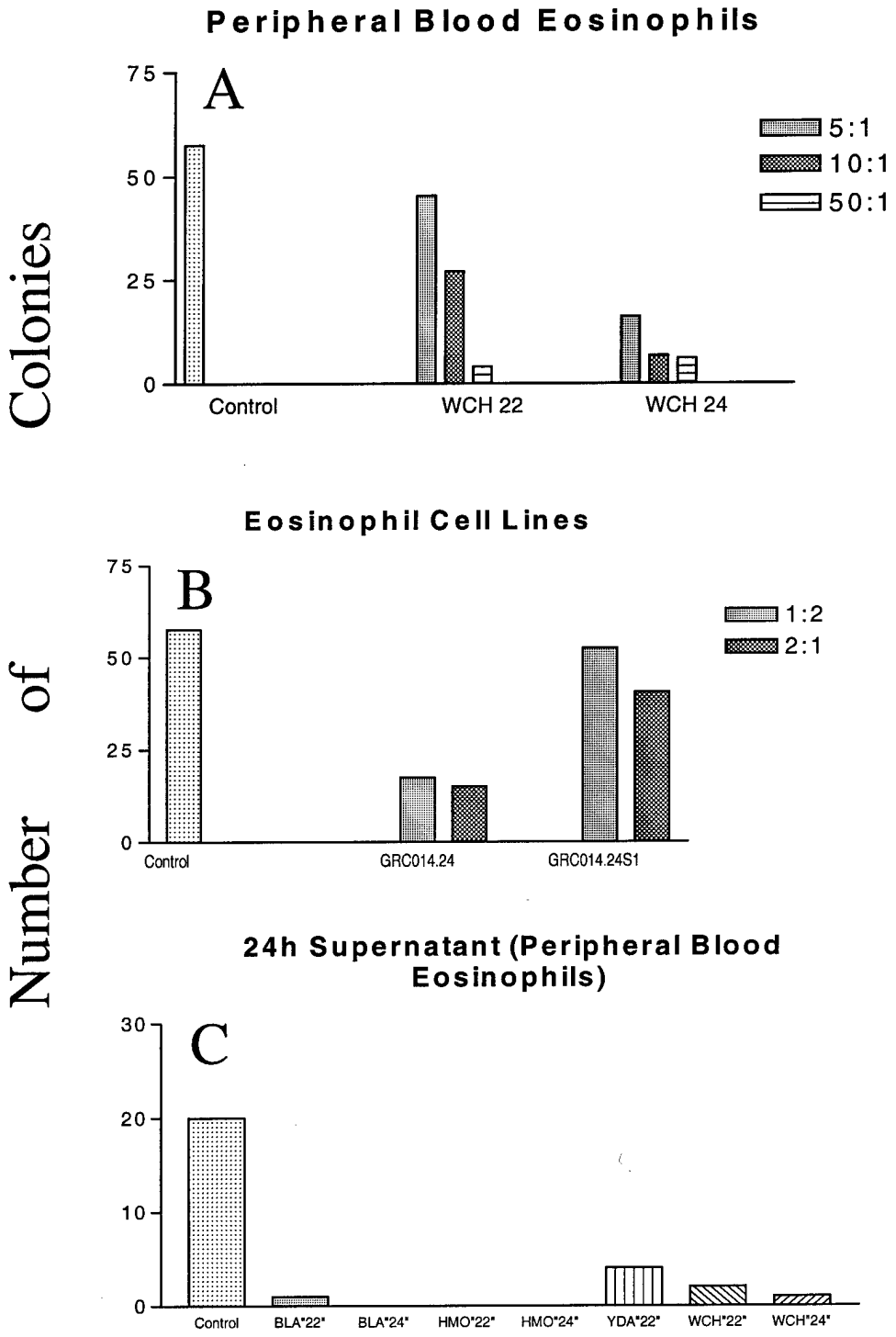
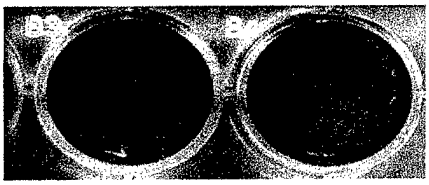
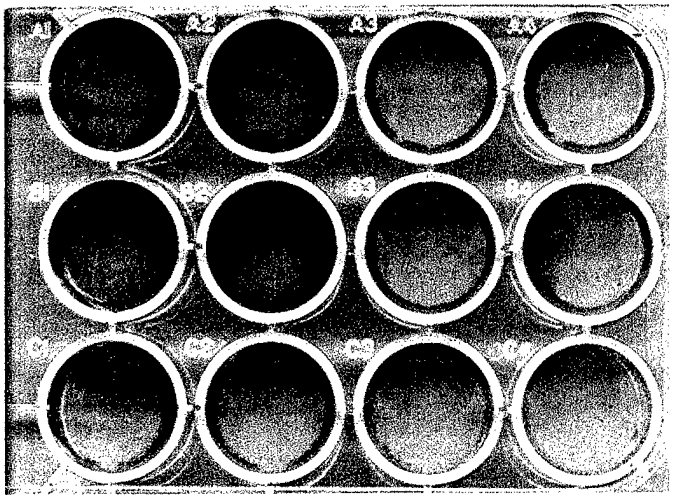


Fig. 7. PC-3 cells were seeded into duplicate and sometimes triplicate wells of a 6-well plate at 100 cells per well. After 24hr incubation eosinophils [fresh peripheral blood eosinophils (panel A); eosinophil cell lines (panel B)], and cultured supernatants (panel C) from both peripheral blood eosinophils (WCH 22 and WCH 24) and eosinophil cell line (GRC.014.24.2) and the plates were further incubated for 10 days. The plates were then harvested, stained with H & E and the colonies counted manually.

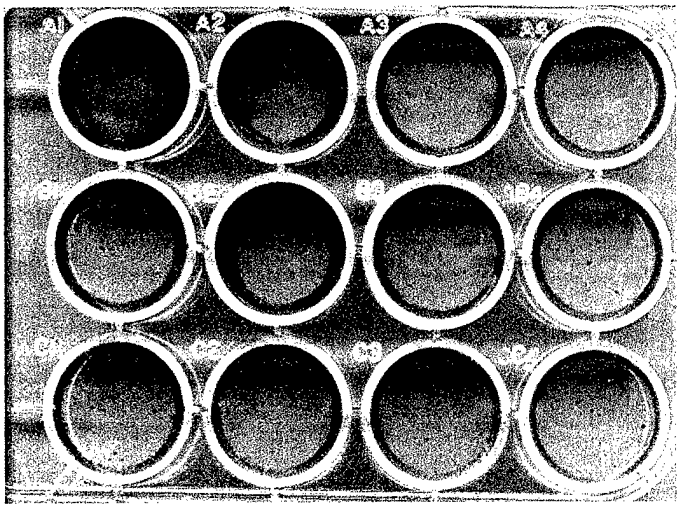
Fig. 8 Eosinophil Cell Lines Inhibit DU145 Cell Growth In Vitro



Control



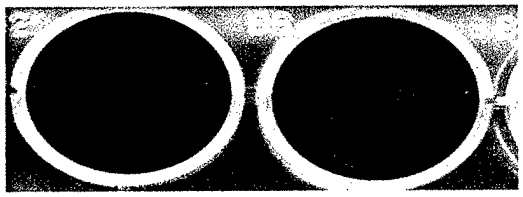
A1-2: BJA060"22":DU145, 1:1
B1-2: BJA060"22":DU145, 10:1
C1-2: BJA060"22":DU145, 25:1
A3-4: BJA060"22"+IL-5:DU145, 1:1
B3-4: BJA060"22"+IL-5:DU145, 10:1
C3-4: BJA060"22"+IL-5:DU145, 25:1



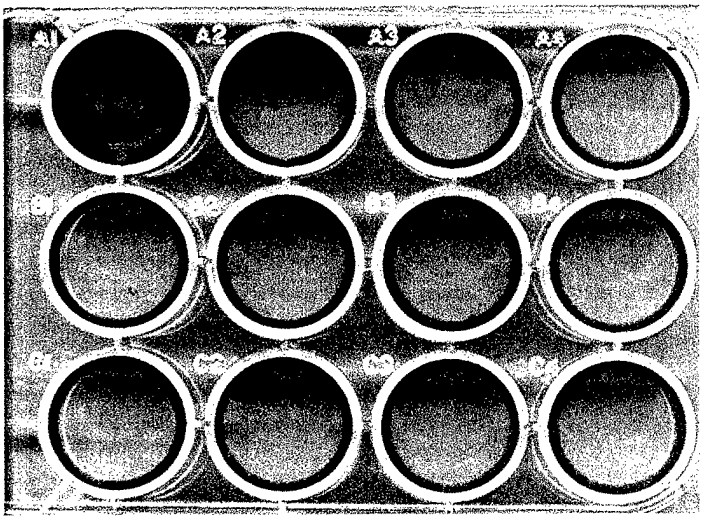
A1-2: GRC014"24":DU145, 1:1
B1-2: GRC014"24":DU145, 10:1
C1-2: GRC014"24":DU145, 25:1
A3-4: GRC014"24"+IL-5:DU145, 1:1
B3-4: GRC014"24"+IL-5:DU145, 10:1
C3-4: GRC014"24"+IL-5:DU145, 25:1

Fig. 8. DU145 prostate cells were seeded into duplicate wells of a 6-well plate at 1.5×10^5 cells/well and incubated overnight at 37°C. IL-5 treated and untreated eosinophil cell lines were added at E:T ratios 1:1, 10:1, and 25:1. The plates were incubated for an additional 24-48hr. Effector cells were removed, the plates were washed 3x with PBS then fixed and stained with hematoxylin and eosin. The entire plate or individual wells were then scanned into power point
for presentation.

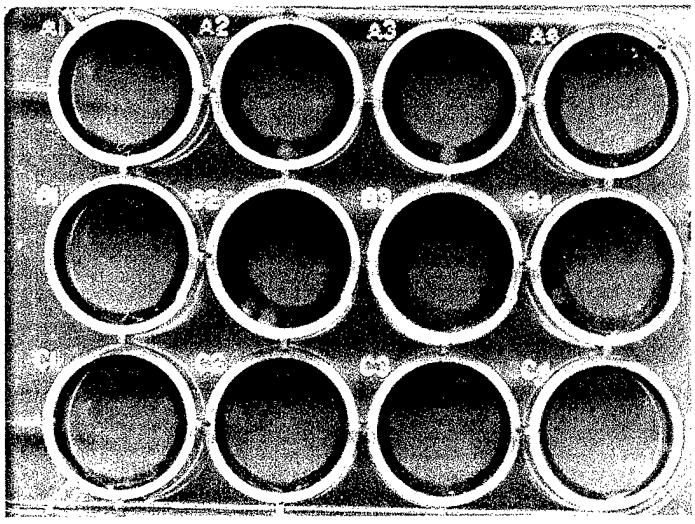
Fig. 9. 24hr Cultured Supernatants Inhibit DU145 Cell Growth In Vitro



Control



A1-2: BLA"22"
A3: BLA"24"
A4: HMO"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 9. DU145 cells ($1.5x10^5$) were treated with 24hr. cultured supernatants from various donor peripheral blood eosinophil subpopulations. Cells were also treated with IL-4 and TNFα at 10, 50 and 100ng/ml. The plates were stained with H & E and scanned into power point for presentation.

Fig. 10. DU145 Colony Inhibition by Eosinophil Cell Lines and Cultured Supernatants

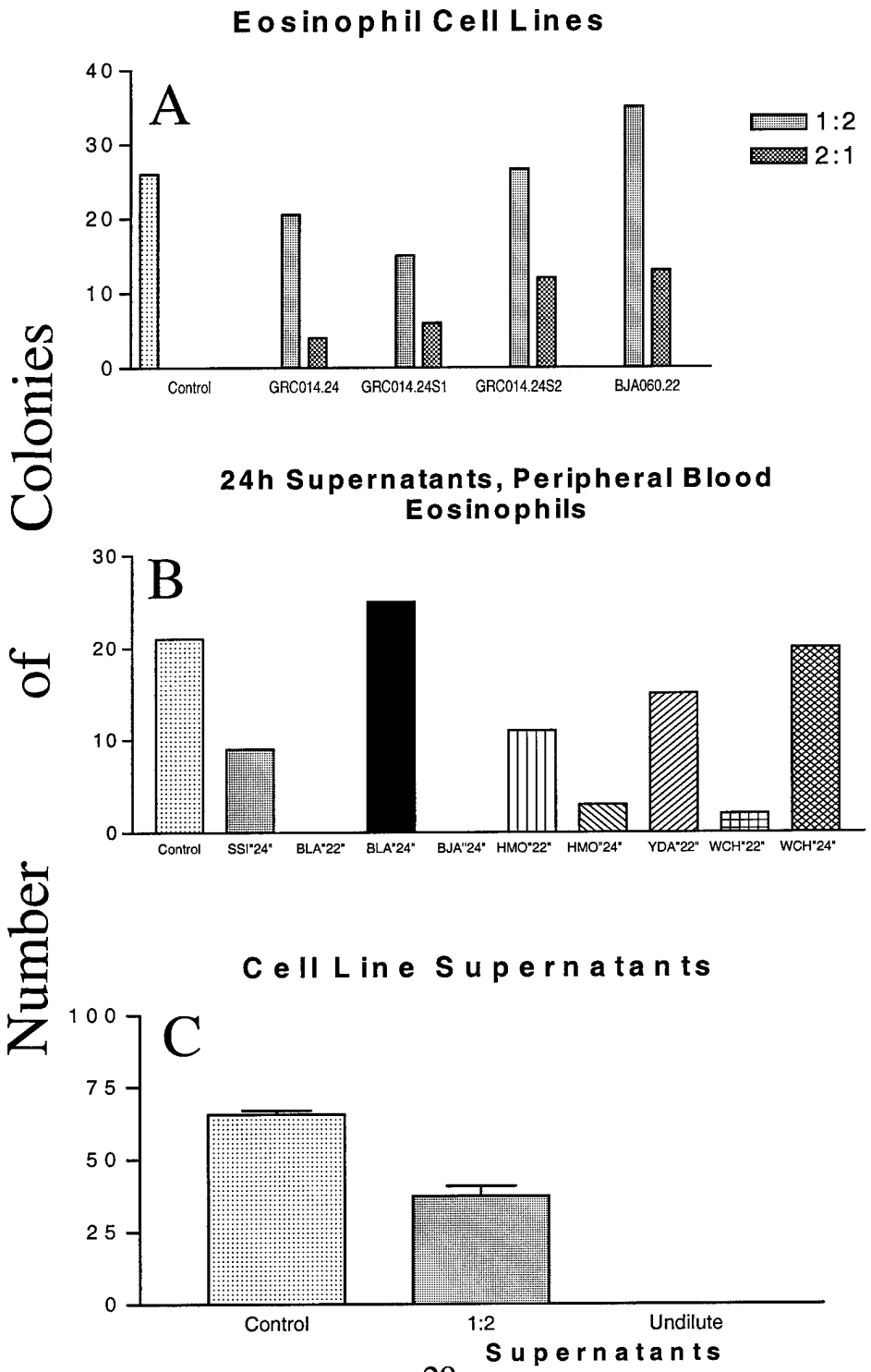
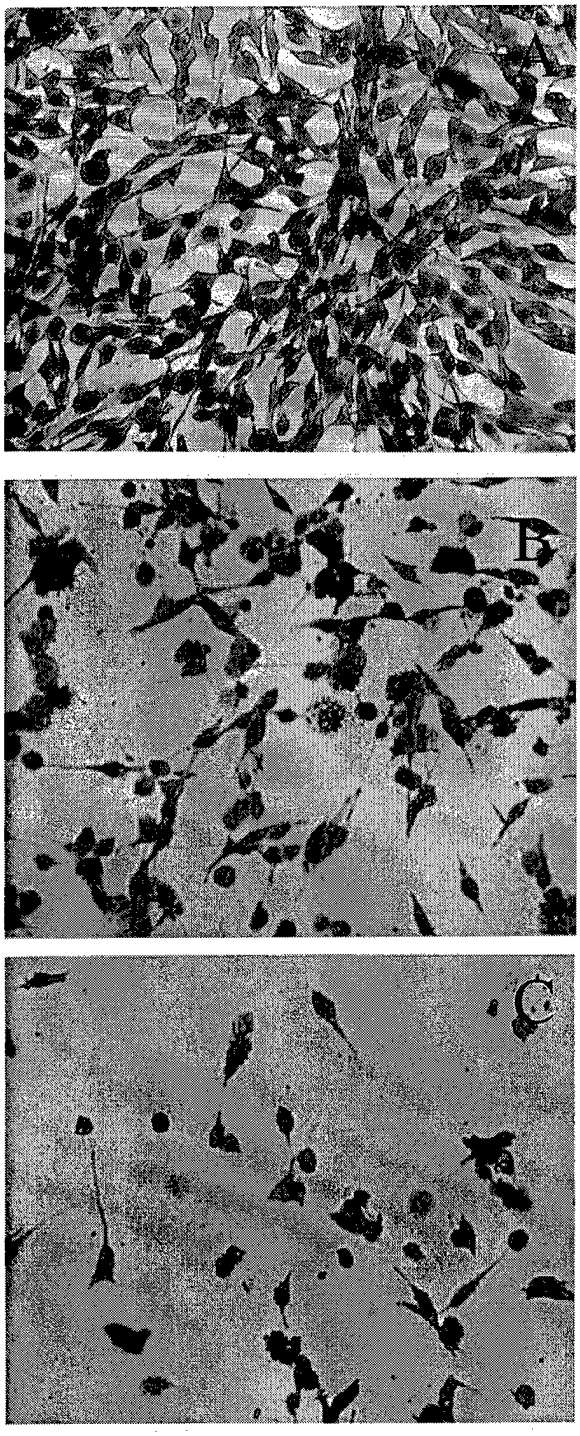


Fig. 10. DU145 cells were seeded into 6-well plates at 100 cells/well and incubated overnight at 37°C. At 24hrs. eosinophil cell lines were added at E:T ratios of 1:2 and 2:1. The plates were then cultured for 10 days at 37°C, afterwhich the plates were stained with H & E and the colonies counted (Panel A). Parent Tumor cells were also incubated with supernatants (Panel B) from cultured eosinophils from various donors and also from a cultured eosinophil cell line (Panel C).

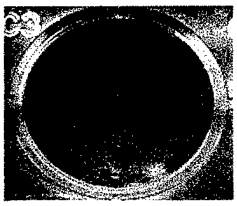
Fig. 11. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines



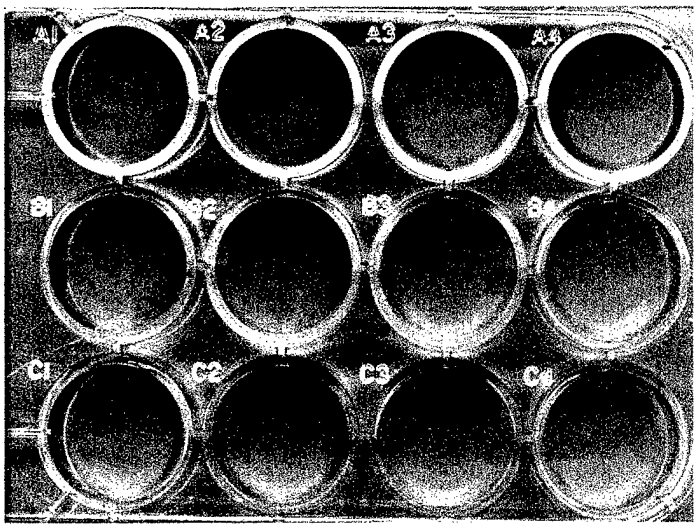
Photomicrograph

Fig. 11. HPC1 cells were seeded into the wells of a 12-well tissue cluster plate at $1.5x10^5$ cells/well. Eosinophil cell lines (IL-5 treated and untreated) were added 24hrs. later the E:T ratio of 10:1. The plates were further incubated for 24-48hr., then harvested and photomicrographs taken.

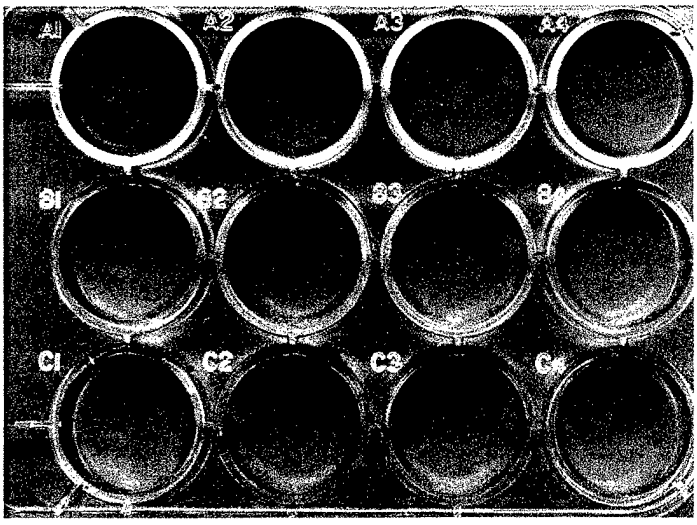
Fig.12. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines



Control



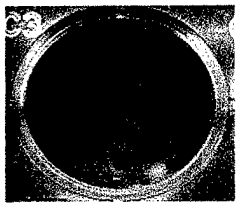
A1-2: GRC014"24":HPCI, 1:1
B1-2: GRC014"24":HPCI, 10:1
C1-2: GRC014"24":HPCI, 25:1
A3-4:GRC014"24"+IL-5:HPCI, 1:1
B3-4:GRC014"24"+IL-5:HPCI, 10:1
C3-4:GRC014"24":+IL-5HPCI, 25:1



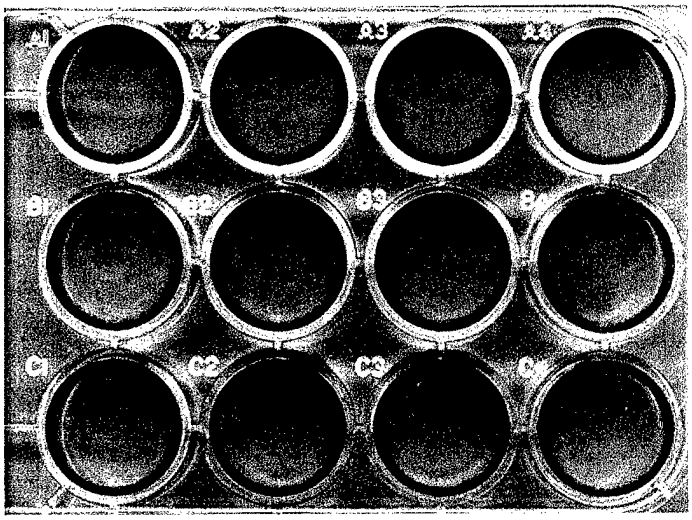
A1-2: BJA060"22":HPCI, 1:1
B1-2: BJA060"22":HPCI, 10:1
C1-2: BJA060"22":HPCI, 25:1
A3-4:BJA060"22"+IL-5:HPCI, 1:1
B3-4:BJA060"22"+IL-5:HPCI, 10:1
C3-4:BJA060"22"+IL-5:HPCI, 25:1

Fig. 12. HPCl cells were seeded into duplicate wells of a 12-well tissue culture plate similarly to that described in fig. 11. Effector cells (IL-5 treated and untreated) were added at E:T ratios of 1:1, 10:1 and 25:1. The plates were stained and scanned into power point.

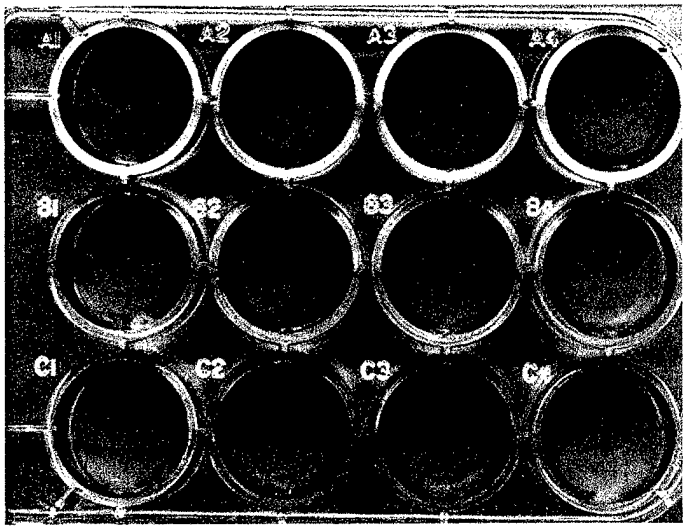
Fig 13. 24hr Eosinophil Cultured Supernatants Inhibit HPCI Cell Growth In Vitro



Control



A1-2: BLA"22"
A3: BLA"24"
A4: HMO"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 13. HPC1 cells ($1.5x10^5$/well) were cultured for 24hrs. were incubated for an additional 24-48hrs with cultured eosinophil supernatants (Panel A) and with IL-4 and TNFα (Panel B). The plates were harvested, stained with H & E and scanned into power point.

Table 1. CYTOKINE CONCENTRATIONS IN 24HR EOSINOPHIL CULTURE SUPERNATANTS (pg/ml)

Donor	IL-4		IL-5		TNFα		GM-CSF	
	22	24	22	24	22	24	22	24
1	>1000	>1000	440	435	50	63	0	0
2	316	3	0	0	100	56	0	0
3	>1000	631	0	0	50	16	0	0
4	>1000	0	nt	nt	129	200	nt	nt
5	200	20	0	0	100	224	nt	nt
6	8	>1000	0	186	10	7.9	450	450

Table 1. 24hr conditioned supernatants were tested for cytokines IL-4, IL-5, TNFα and GM-CSF using commercial enzyme linked immunoassay kits.

Dennery, Morice 352522

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Keywords: African American, Cell culture, Prostate cancer

A NOVEL PRIMARY PROSTATE CANCER CELL LINE DERIVED FROM AN AFRICAN AMERICAN PATIENT.

Chiledum Ahaghotu, Washington, D.C.; Josephine Marshalleck, Washington, D.C.; Morice Dennery, Washington, D.C.; Theresa Vaughn, Washington, D.C.; Ibouraima Laniyan, Washington, D.C.; Aaron Jackson, Washington, D.C.; and Paulette Furbert-Harris, Washington, D.C..

Introduction and Objectives. Current epidemiological data suggests that African American prostate cancer patients tend to present with relatively aggressive phenotype compared to other ethnic groups in the U.S. The disparity probably represents genetic and environmental factors, both of which warrant in vitro studies. We present preliminary data on a primary prostate cancer cell culture system derived from an African American patient.

Methods. Malignant prostate tissue was obtained transurethrally from an African American patient presenting with hormone-refractory local tumor progression following radical retropubic prostatectomy. Tissue samples were submitted for H&E and immunohistochemical staining with PSA, PAP, CAM 5.2/AE-1, CK 7, 20, 903 and Lewis X antibodies. Remaining tissue was transported in Hank's balanced salt solution and processed by standard isolation and primary culture techniques. Briefly, collagenase-digested single-cell suspensions were dispensed into six-well plates coated with type I rat tail collagen. The plates underwent undisturbed CO_2 incubation at 37° C in prostate-specific serum-free culture media for 96 hours. Refeeding was done every 3 to 4 days. Actively proliferating primary cultures (1:2 split) were immortalized by Human Papilloma viral transfection using the LXSN16E6E7 retroviral construct. Confluent flasks were harvested and cell pellets were paraffin-embedded and submitted for immunohistochemical staining.

Results. Standard H&E staining of original prostate tissue samples demonstrated sheets of anaplastic cells with no evidence of residual stromal or epithelial elements. Immunohistochemical staining was positive for PSA, PAP, and CAM 5.2/AE-1 (cytokeratins 39, 40, 43, 48, 50, 50.6 kd) and negative for Lewis X, CK 7, 20, and 903 (cytokeratins 46, 54, 56, 56.5, 58, 68 kd) antibodies. Despite marked similarities in histologic appearance between original tissue samples and primary cell culture, immunohistochemical staining of primary cells with previously described antibodies were negative.

Conclusions. Our laboratory presents preliminary findings on the first reported primary prostate cancer cell lined derived from an African American patient with hormone refractory disease. Further characterization is in progress.

Source of funding: Department of Defense:DAMD17-98-1-8485, Howard University Cancer Center.

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